PROMOTER-DEBUGGING MODULE: LAB NOTEBOOK WEEKLY SUMMARY

Week 1 (1/6/2015-5/6/2015)

1. Summer training, mainly for recalling the basic techniques

2. Discussion for the plan of the main project

Week 2 (8/6/2015-12/6/2015)

1. Transformation of pSB1C3-BBa_I0500, pSB1C3-BBa_B0015, pSB1C3-BBa_B0030, pSB1C3-BBa_K381001 and pSB1C3-BBa_E0840

2. Inoculation of pSB1C3-BBa_I0500, pSB1C3-BBa_B0015, pSB1C3-BBa_B0030, pSB1C3-BBa_K381001 and pSB1C3-BBa_E0840

3. Plasmid extraction of pSB1C3-BBa_I0500, pSB1C3-BBa_B0015, pSB1C3-BBa_B0030, pSB1C3-BBa_K381001 and pSB1C3-BBa_E0840

4. PCR cloning of *phoR* from *E. coli* strain DH10B

5. Phosphorylation and annealing of P_{phoA}

6. Digestion of pSB1C3-BBa_J04450

7. Ligation of *phoR* with pSB1C3, P_{phoA} with pSB1C3 and pSB1C3-BBa_I0500 with pSB1C3-BBa_B0030

8. Colony PCR for identifying candidate colonies for pSB1C3-*phoR*

Week 3 (15/6/2015-19/6/2015)

1. Transformation of pSB1A2-BBa_B0030, pSB1A2-BBa_E0840, pSB1AK3-BBa_B0015

2. PCR reaction for identifying candidate colonies for pSB1C3-*phoR* and pSB1C3-BBa_I0500-B0030

3. Phosphorylation and annealing of P_{phoA}

4. Digestion of pSB1C3-BBa_J04450

5. Sequencing of pSB1C3-*phoR*

Week 4 (22/6/2015-26/6/2015)

1. Colony PCR for checking the identity of pSB1C3-*phoR* candidate 7 and 8 by VF_2 and VR primers

2. Colony PCR for checking the identity of pSB1C3-*phoR* candidate 7 and 8 by F_{phoR} and VR primers

3. Restriction check of pSB1C3-*phoR* candidate 7 and 8 using BstEII

4. Colony PCR for checking the identity of pSB1C3-P_{phoA} candidate 1 and 3 by VF_2 and VR primers

5. Colony PCR for checking the identity of pSB1C3-P_{phoA} candidate 1 and 3 by V_{PphoA} and VR primers

6. Restriction check of pSB1C3-P_{phoA} candidate 1 and 3 using PvuII

7. PCR cloning of *nsrR* from *E. coli* strain DH10B

8. Ligation of *nsrR* with pSB1C3

9. Transformation of pSB1C3-*nsrR*

Week 5 (29/6/2015-3/7/2015)

1. New plan for the constructs: instead of having constructs of pSB1C3-*nsrR* and pSB1C3-*phoR* for sequencing, rather, ligate *nsrR* and *phoR* with pSB1C3-BBa_B0030, respectively, while no major change for P_{phoA}.

2. Digestion of pSB1C3-BBa_B0030.

3. PCR cloning of *nsrR* and *phoR* from *E. coli* strain DH10B.

4. Digestion of PCR product of *nsrR* and *phoR.*

5. Ligation of pSB1C3-BBa_B0030 with *nsrR* and *phoR* respectively.

6. Digestion of pSB1C3-BBa_J04450.

7. Overnight ligation of P_{phoA} with pSB1C3 at 16^{o}C.

8. Transformation of pSB1C3-BBa_B0030-*nsrR*, pSB1C3-BBa_B0030-*phoR* and pSB1C3-P_{phoA}.

9. Colony PCR for checking the identity of pSB1C3-BBa_B0030-nsrR, pSB1C3-BBa_B0030-*phoR* and pSB1C3-P_{phoA} candidate colonies. Identified candidate 1 and 5 of pSB1C3-BBa_B0030-*phoR* and candidate 1-17 of pSB1C3-P_{phoA}.

10. Confirmed the identity of candidate 1 and 5 of pSB1C3-BBa_B0030-*phoR* by restriction check using PvuII, ready for sequencing.

11. Digestion of pSB1C3-BBa_B0030

12. Ligation of pSB1C3-BBa_B0030 with *nsrR*.

13. Transformation of pSB1C3-BBa_B0030-*nsrR*.

13. New plan for P_{phoA} , instead of having the construct of pSB1C3-P_{phoA} for sequencing, rather, ligate P_{phoA} with pSB1A2-BBa_E0840 for sequencing.

14. Digestion of pSB1A2-BBa_E0840

15. Overnight ligation of pSB1A2-BBa_E0840 with P_{phoA} at 16^{o}C.

Week 6 (6/7/2015-10/7/2015)

1. Send pSB1C3-BBa_B0030-*phoR* candidate 5 for sequencing.

2. Ligation of pSB1C3-BBa_B0030 with *nsrR*.

3. Transformation of pSB1C3-BBa_B0030-*nsrR*.

4. Colony PCR of pSB1C3-BBa_B0030-*nsrR* candidate 1-22.

5. Identified candidate 3 and 13 of pSB1C3-BBa_B0030-*nsrR* by colony PCR.

6. Restriction check of pSB1C3-BBa_B0030-*nsrR* candidate 3 and 13 by ScaI.

7. Identified pSB1C3-BBa_B0030-*nsrR* candidate 13 by restriction check.

8. Send pSB1C3-BBa_B0030-*nsrR* candidate 13 for sequencing.

9. Transformation of pSB1A2-P_{phoA}-BBa_E0840.

10. Identified pSB1A2-P_{phoA}-BBa_E0840 candidates by restriction check using PvuII.

11. Miniprep of pSB1C3-BBa_B0030-*phoR* candidate 5, 7, 8, pSB1C3-BBa_B0030-*nsrR* candidate 3 and

13, pSB1A2-P_{phoA}-BBa_E0840 candidate 1-5.

12. Ligation of pSB1A2-BBa_E0840 with P_{phoA}.

13. Send pSB1A2-P_{phoA}-BBa_E0840 candidate 5 for sequencing.

14. Sequencing result of pSB1C3-BBa_B0030-*phoR* candidate 5 showed missing of BBa_B0030.

15. Inoculation and streak pSB1C3-BBa_B0030, pSB1AK3-BBa_B0015

Week 7 (13/7/2015-17/7/2015)

1. Send pSB1C3-BBa_B0030, pSB1C3-BBa_B0030-*phoR* candidate 1, pSB1A2-P_{phoA}-BBa_E0840 for sequencing.

2. Confirmed pSB1A2-*PphoA*-BBa_E0840 from the sequencing result.

3. From sequencing result of pSB1C3-BBa_B0030, it showed extra nucleotides before and after the BBa_B0030 sequence, which is inconsistent with the sequence from the iGEM Parts Registry.

4. Transformation of pSB1C3-BBa_B0032.

5. Miniprep of pSB1C3-BBa_B0032 and pSB1C3-BBa_B0030.

6. Digestion of pSB1C3-BBa_B0032.

7. Ligation of pSB1C3-BBa_B0032 with *phoR* and *nsrR* separately.

8. Transformation of pSB1C3-BBa_B0032-*nsrR* and pSB1C3-BBa_B0032-*phoR*.

9. Colony PCR of pSB1C3-BBa_B0032-*nsrR* candidate 1-8 and pSB1C3-BBa_B0032-*phoR* candidate 1-8.

10. Restriction check of pSB1C3-BBa_B0032-*nsrR* candidate 5 and pSB1C3-BBa_B0032-*phoR* candidate 6.

11. Glycerol stock for pSB1AK3-BBa_B0015.

12. Functional assay for P_{phoA} and P_{yeaR}

12. Characterization of P_{phoA} and P_{yeaR}.

13. Prepared modified M9 minimal medium for P_{yeaR-} characterization with NH_4Cl being replaced by MOPS salt for testing the viable range.

14. Prepared modified M9 minimal medium for P_{phoA} characterization with phosphate being replaced.

Week 8 (20/7/2015-24/7/2015)

1. Digestion of *nsrR, phoR* PCR product and pSB1C3-BBa_I0500-B0030.

2. Ligation of *nsrR* and *phoR* with pSB1C3-BBa_I0500-B0030 separately.

3. Transformation of pSB1C3-BBa_I0500-B0030-*nsrR* and pSB1C3-BBa_I0500-B0030-*phoR.*

4. Colony PCR for identifying candidates of pSB1C3-BBa_I0500-B0030-*nsrR* and pSB1C3-BBa_I0500-B0030-*phoR.*

5. Functional assay of pSB1C3-BBa_K381001 with 0mM and 20mM KNO_3 .

6. Characterization of pSB1C3-BBa_K381001 with series of 0mM, 5mM, 10mM, 15mM, 20mM, 50mM, 100mM KNO_3..

7. Characterization of pSB1C3-BBa_K381001 with series of 0mM, 2mM, 4mM, 6mM, 8mM, 10mM KNO_3.

8. Ligation of *nsrR* and *phoR* with pSB1C3-BBa_I0500-B0030 separately. (new)

9. Transformation of pSB1C3-BBa_I0500-B0030-*nsrR* and pSB1C3-BBa_I 0500-B0030-*phoR.* (new)

10. Innoculate pSB1A2-P_{phoA}-BBa_E0840 in M9 minimal medium with 0μM, 10μM, 500μM and 1000μM of PO_4 (Using Tris hydrochloride to replace the original PO_4 in M9)

11. Check OD and GFP expression

12. Digestion and gel purification of pSB1A2-BBa_E0840 (for ligating with pSB1A2-BBa_J23101 to act as a positive control)

Week 9 (27/7/2015-31/7/2015)

1. Characterization of pSB1C3-BBa_K381001 in M9 minimal medium with 0μM, 20μM, 200μM, 2000μM KNO_3.

2. Characterization of pSB1C3-BBa_K381001 in LB for 3 trials with 0mM, 2mM, 4mM, 6mM, 8mM, 10mM KNO_3. (Followed directly the protocol from BCCS-Bristol 2010)

As the constructs of pSB1C3-BBa_I0500-B0030-*nsrR* and pSB1C3-BBa_I0500-B0030-*phoR* were stuck for 2 months in the cloning process, some trouble-shoots were adopted as follows: (point 1-9)

3. PCR cloning of *nsrR* and *phoR* from DH10B, with 4 tubes of 50μl reaction.

4. PCR purification using small column, 5μl after 5μL for eluting the sample.

5. Digestion of *nsrR* and *phoR* with XbaI and PstI-HF digestion enzymes in 37^oC incubator for 2 hrs.

6. PCR purification using small column, 5μl after 5μL for eluting the sample.

7. Ligation of *nsrR* with pSB1C3-BBa_I0500-B0030 and *phoR* with pSB1C3-BBa_I0500-B0030 for 3 hrs in 16^oC incubator.

8. Transformation of pSB1C3-BBa_I0500-B0030-*nsrR* and pSB1C3-BBa_I0500-B0030-*phoR*.

9. Colony PCR of pSB1C3-BBa_I0500-B0030-*nsrR* candidate 1-26 and pSB1C3-BBa_I0500-B0030-*phoR* candidate 1-26.

10. Characterization of pSB1A2-P_{phoA}-BBa_E0840 in M9 minimal medium with 0μM, 30μM, 50μM, 100μM, 150μM, 200μM, 250μM PO_4^{3-}.

11. Digestion of pSB1A2-BBa_J23101-J61002 (constitutive promoter-RFP reporter)

12. Ligation of BBa_J23101 (constitutive promoter) and BBa_E0840(GFP reporter)

13. Streak plates for pSB1A2-P_{phoA}-BBa_E0840 candidate 5, pSB1A2-E0840 and pSB1A2-BBa_J23101-E0840

Week 10 (3/8/2015-7/8/2015)

1. Restriction check on pSB1C3-BBa_I0500-B0030-*nsrR* candidate 19-23, and 26.

2. Sent pSB1C3-BBa_I0500-B0030-nsrR candidate 23 for sequencing

3. Characterization of pSB1C3-BBa_K381001 using LB medium for trial 4-6 with concentration gradient of 0, 2, 4, 6, 8, 10, 15, 20mM KNO_3

4. Characterization of pSB1C3-BBa_K381001 using M9 minimal medium for trial 1-3 with concentration gradient of 0, 2, 4, 6, 8, 10mM KNO_3

5. PCR cloning of *phoR* from DH10B

6. Digestion of *phoR*.

7. Ligation of phoR with pSB1C3-BBa_I0500-B0030 (P_{bad}-RBS)

8. Transformation of pSB1C3-BBa_I0500-B0030-*phoR*.

9. Colony PCR of pSB1C3-BBa_I0500-B0030-*phoR*.

10. Characterization of pSB1A2-BBa_P_{phoA}-E0840.

Week 11 (10/8/2015- 14/8/2015)

1. Sent pSB1C3-BBa_I0500-B0030-nsrR candidate 23 for sequencing

2. Characterization of pSB1C3-BBa_K381001 using LB medium for trial 8 with concentration gradient of 0, 2, 4, 6, 8, 10, 15, 20mM KNO_3

3. Characterization of pSB1C3-BBa_K381001 using M9 minimal medium for trial 4-6 with concentration gradient of 0, 2, 4, 6, 8, 10mM KNO_3

4. Characterization of pSB1C3-BBa_K381001 using M9 minimal medium for trial 1-5 with concentration gradient of 0, 20, 200, 500, 1000, 2000µM KNO_3

5. Restriction check of pSB1C3-BBa_I0500-B0030-*phoR* candidate 6-10 using enzyme BstEII

6. Sent pSB1C3-BBa_I0500-B0030-*phoR* candidate 9 for sequencing

Week 12 (17/8/2015- 23/8/2015)

1. Ligate pSB1C3-BBa_I0500-B0030-*nsrR* with pSB1C3-BBa_K381001

2. Identifying correct candidates for pSB1C3-BBa_I0500-B0030-*nsrR*-K381001 by restriction check and colony PCR.

3. Characterization of P_{yeaR} promoter in M9 minimal medium with concentration of 0-500µM, and in LB with concentration of 0-50mM.

4. Gibson Assembly on pSB1C3-BBa_I0500-B0030-*phoR*-B0015 construct.

5. Identifying candidates for pSB1C3-BBa_I0500-B0030-*phoR*-B0015 construct using colony PCR.

6. Characterization on P_{phoA} promoter.

7. Characterization on P_{pboBR} promoter.

8. Characterization on P_{amn} promoter.

Week 13 (24/8/2015-30/8/2015)

1. 1. Ligate pSB1C3-BBa_I0500-B0030-*nsrR* with pSB1C3-BBa_K381001

2. Identifying correct candidates for pSB1C3-BBa_I0500-B0030-*nsrR*-K381001 by restriction check and colony PCR.

3. Confirmed pSB1C3-BBa_I0500-B0030-*nsrR*-K381001 candidate 6 is correct.

3. Characterization of P_{yeaR} promoter in M9 minimal medium with concentration of 0-500µM.

4. Gibson Assembly on pSB1C3-BBa_I0500-B0030-*phoR*-B0015 construct.

5. Identifying candidates for pSB1C3-BBa_I0500-B0030-*phoR*-B0015 construct using colony PCR and restriction check.

6. Functionality assay on the pSB1C3-BBa_I0500-B0030-*nsrR*-K381001, the debug method with P_{yeaR} promoter.

7. Characterization on P_{phoA} promoter.

8. Characterization on P_{pboBR} promoter.

9. Characterization on P_{amn} promoter.

Week 14 (31/8/2015-6/9/2015)

1. Characterization on the pSB1C3-BBa_I0500-B0030-*nsrR*-K381001, the debug method with P_{yeaR} promoter with M9 minimal medium from 0- 2mM nitrate and 10mM arabinose concentration.

2. Characterization on P_{phoA} promoter.

3. Characterization on P_{pboBR} promoter.

4. Characterization on P_{amn} promoter.